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This document is a progress report which describes the results from the first of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of metabolic heat production during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fuelling metabolism; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to evaluate the subsequent effects on metabolic rate and body temperature rgulation during cold stress. Female subjects were immersed to the neck in 18 degC water for up to 90 minutes. Their metabolic rate increased to about 3 times resting levels, similar to what what reported for males. About 40% of metabolism was fueled by carbohydrate oxidation. Muscle glycogen was a significant contributor to metabolism. The protocol was not successful in manipulating glycogen availability.

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2. ABSTRACT

This document is a progress report which describes the results from the first of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of metabolic heat production during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling metabolism; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on metabolic rate and body temperature regulation during cold stress. Female subjects were immersed to the neck in 18°C water for up to 90 minutes. Their metabolic rate increased to about 3 times resting levels, similar to what was reported previously for male subjects. About 40% of the metabolic heat production during immersion was fueled by oxidation of carbohydrates, somewhat less than what was reported for male subjects. Also, as reported earlier for males, muscle glycogen decreased significantly during the immersion suggesting that it is a significant constituent of the carbohydrate component of metabolic heat production during shivering. The implications of manipulating glycogen availability in females could not be adequately addressed because the protocol did not result in the desired changes in muscle glycogen prior to immersion.

3. INTRODUCTION

Military units operate in cold air and cold water environments, and the associated training or missions can result in personnel being faced with life-threatening situations if they are ill-equipped or unprotected. As demonstrated by the recent winter crash in the Canadian Arctic of a military aircraft carrying infantry personnel, rescue can be delayed for days even when the precise location of survivors is known (de Groot, 1994). Cold water immersion hypothermia recently caused the deaths during training of US Army Rangers (Fort Benning, 1995). In light of such potential emergencies the prediction of survival time (*ST*) in the cold, defined in this document as the elapsed time until the onset of lethal hypothermia, is essential to meet the needs of Search and Rescue authorities. Such predictions are also useful in the analysis of strategic human factors demands of military operations in the cold, to prepare for contingencies of such operations, and to evaluate the potential benefits of equipment/clothing designed to protect the soldier from the cold.

An understanding of *ST* in healthy, sedentary, non-traumatized individuals is based in the following relationships. Once the protective insulation of available shelter or clothing is maximized, cold-stressed humans elevate metabolic heat production ($\dot{M}$) by shivering in an attempt to balance heat loss. Existing models of *ST* in cold air or cold water are based on observations of factors which affect $\dot{M}$ and the rate of heat loss from the body. In such models $\dot{M}$ increases as a function of temperature signals from the core and skin. When cold exposure is too severe for $\dot{M}$ to balance heat loss, *ST* is largely determined by the rate of heat loss from the body. Where there is a balance between $\dot{M}$ and heat loss, *ST* is limited by the endurance time for shivering.

The physiological factors characterizing $\dot{M}$ are relatively complex. Until about a decade ago there was very little empirically based information available in this regard for human subjects. Research has demonstrated that the relationship

between *ST*, $\dot{M}$, and heat loss is affected by the extent of the muscle mass involuntarily recruited during shivering (Bell et al., 1992), connective heat transfer during cold stress (Tikuisis et al., 1991), muscle substrate availability (Jacobs et al., 1994), the type and quantity of substrate oxidized by shivering musculature (Vallerand and Jacobs, 1989), and body composition (Tikuisis et al., 1988). Our research during the last decade has focused on such factors with the objective of generating sufficient knowledge to improve the predictive modelling of *ST* in the cold. A brief review of this research follows.

By measuring the electrical activity of many muscle groups simultaneously during cold-induced shivering, we demonstrated that several large muscle groups are recruited and contract at relatively low intensities that are less than 20% of their maximum force generating capabilities (Bell et al., 1992). Since so many muscle groups are involved in shivering, the sum total of their contractile activities can result in a four or five-fold increase in metabolic rate, and heat production.

Much of our attention has been directed towards the substrates that are used by skeletal muscle to increase heat production during shivering. For example, Vallerand et al. (1988) administered a clinical glucose tolerance test to subjects who were sitting in either cold air or at a comfortable temperature for two hours. These data were the first to show in humans that glucose is eliminated more rapidly from the circulation during cold exposure, presumably to provide more available substrate to fuel the increase in metabolic rate. It is also noteworthy that this more rapid uptake of glucose during cold exposure occurs with lower insulin levels in the cold compared to warm temperatures.

We subsequently continued to attempt to quantify the rates of substrate oxidation of fat, carbohydrate and protein in humans during cold exposure with indirect calorimetric techniques. As one might presume, the increase in metabolic rate during shivering is caused by increases in oxidation of both fat and carbohydrate, but the relative increase in the rate of substrate oxidation caused by shivering is greatest for carbohydrates (Vallerand and Jacobs, 1989). In resting subjects exposed to either cold air or cold water, carbohydrates and fat contribute

approximately equally to heat production (Martineau and Jacobs, 1991; Vallerand and Jacobs, 1989). From a strategic point of view, this finding seems unfortunate because the body's availability of carbohydrates is quite limited compared to the abundant fat and protein stores. We were already aware of the well established positive relationship between muscle glycogen concentration and endurance exercise performance of skeletal muscle and speculated that there may be a similar detrimental effect caused by muscle glycogen depletion on another form of muscle contraction, i.e. shivering and the associated heat production.

We therefore carried out a series of studies on male subjects immersed in 18°C water. The subjects were removed from the water when their rectal temperature reached 35.5° C. Biopsies were taken from the thigh muscle before and after the immersion to evaluate the changes in glycogen as a result of the water immersion (Martineau and Jacobs, 1988). In another study muscle glycogen concentrations were manipulated prior to water immersion by appropriate dietary and exercise protocols (Martineau and Jacobs, 1989); the purpose of these studies was to evaluate the effects of very low and very high glycogen levels on metabolic heat production during the water immersion.

Metabolic rate during cold water immersion, expressed as oxygen consumption, increases to values that are usually around 4 or 5 times normal resting metabolic rate. Infrequently we have observed individuals who exhibit somewhat higher values, 6– or 7 times resting values. Our initial studies suggested that part of this increase in metabolic rate is fueled by muscle glycogen, as all of the subjects demonstrated a decrease in leg glycogen concentration after the water immersion (Martineau and Jacobs, 1988). The second objective of these experiments was to evaluate the effects of manipulating the pre-immersion glycogen levels on heat production during cold water immersion. Our manipulations did result in the subjects entering the water on one trial with muscle glycogen levels that were only about 50% of normal, and on another trial when they were about 150% of normal (Martineau and Jacobs, 1989). The oxygen consumption during the water immersion, was about the same on each trial. The respiratory exchange ratio (RER), however, differed between trials as expected.

Metabolic heat production is calculated based on the combination of RER and oxygen consumption. We observed significantly less metabolic heat production per unit time when the body's carbohydrate stores were depleted compared to the other trials (Martineau and Jacobs, 1989). There was also a significantly more rapid body cooling rate, as reflected by the changes in rectal temperature, when the body had little glycogen stored in its muscles, and presumably also in the liver.

These examples of some of our initial studies were done on subjects resting in cold air or cold water. In light of these findings we hypothesized that the requirement to do physical work superimposed on that cold stress might induce a more rapid breakdown of muscle glycogen than if the same work were done at a comfortable temperature. We therefore had subjects performing either light or heavy exercise once at 9°C air and again on a separate day at 21°C (Jacobs et al., 1985). We found that significantly more glycogen was in fact utilized to do the light exercise in the cold compared to doing the same work at 21°C. There was no difference in glycogen depletion rates, however, for the higher exercise intensities, and this is consistent with earlier observations that the heat production associated with hard exercise is sufficient to offset heat loss to the environment, thus obviating the need for shivering (Hong and Nadel, 1979).

We also carried out investigations of the effects of manipulating the body's circulating fat pools on heat production during cold water immersion. Vallerand and Jacobs (1990) reported that triglycerides infused intravenously were not eliminated more rapidly from the circulation during cold air exposure than during warm air exposure, contrasting with the results for glucose infusion (Vallerand et al., 1988). In another series of experiments, the circulating free fatty acid concentration was manipulated by having our subjects ingest nicotinic acid in the form of niacin pills prior to and during the water immersion (Martineau and Jacobs, 1989b). The effect of the nicotinic acid is to block lipolysis and this effect is demonstrated by the observation that the plasma free fatty acids and glycerol levels were dramatically reduced prior to, and during, the water immersion. Again contrasting with the effects of manipulating the carbohydrate stores, metabolic heat production was virtually unaffected; the proportion of the

total heat production that could be attributed to fat oxidation was significantly reduced, but there was compensation by simply increasing carbohydrate oxidation.

For reasons that are still unclear, carbohydrates seem to be a somewhat preferred substrate during shivering thermogenesis. There are similarities to hard physical exertion in that the body is not able to maintain the same intensity of exertion when carbohydrate stores are depleted, i.e. a shift to a greater reliance on fat oxidation to fuel muscle contraction is not sufficient for the musculature to be able to maintain a high level of exertion, just as body temperature could not be maintained as well when carbohydrate stores were depleted (Martineau and Jacobs, 1989a). We must mention that similar experiments were carried out at USARIEM and they did not detect any significant muscle glycogen utilization during cold water immersion (Young et al., 1989); we can not explain the discrepancies between our studies other than to suggest that perhaps the fact that our subjects were much leaner than those of Young et al. (1989) may be important in this regard.

Gender differences in response to cold stress have been the topic of a limited number of investigations and reviews (Stephenson and Kolka, 1993; Nunneley, 1978; Hayward et al., 1975) . It was reported that women cool faster than men during cold water immersion (Kollias et al., 1974; McArdle et al., 1984; Hessemer and Brück, 1985) , and this is somewhat surprising considering the greater body fat content of the average female. Body temperature changes associated with the menstrual cycle (Graham et al., 1989) , cardiovascular responses to rest and exercise (Stevens et al., 1987; Wagner and Horvath, 1985a,b) are other factors with associated gender differences in response to cold stress. To date potential gender-related physiological differences in responses to cold have not been considered in systematic studies such as those described above, i.e. quantification of the substrates used to fuel $\dot{M}$ during cold stress, nor in the development of ST predictive models, including our own [Tikuisis, 1989; Tikuisis et al., 1988). Specifically, there are established gender differences in the ratio of lean body mass to total body mass and in the proportion of energy derived from carbohydrate or fat metabolism during exercise (Tarnopolsky et al., 1990).

There are, however, studies of gender differences with regard to skeletal muscle metabolism during exercise which suggest that untrained female musculature has an enzymatic profile which is predisposed to greater dependency on lipid metabolism than male muscle tissue (Green et al., 1984) . In male and female subjects matched for their physical training status, exertion at the same relative intensity is fueled by carbohydrate oxidation to a greater extent in males, and by lipid oxidation to a greater extent in females (Tarnopolsky et al., 1990; Phillips et al., 1993; Tarnopolsky et al., 1995) . Although potentially advantageous for endurance exercise, the evidence presented above relating to the importance of carbohydrate oxidation for shivering thermogenesis suggests that less carbohydrate oxidation may be disadvantageous in terms of *ST* in the cold. However, even if the magnitude of the increase in $\dot{M}$ may be less in females than males, the metabolic predisposition favoring lipid oxidation suggests that temperature regulation may not be as negatively influenced when glycogen availability is compromised. In terms of the muscle mass involved in shivering, models of human thermoregulation during cold stress use a fixed value to represent the contribution of the musculature of various body segments to the increase in $\dot{M}$ due to shivering. For example, this constant for the contribution of the trunk has previously only been estimated and ranged from 55-85% (Montgomery, 1974; Stolwijk, 1970; Hancock, 1980) . We recently experimentally determined this value for male subjects to be 71% (Bell et al., 1992), but here again no data are yet available for female subjects. The implications of these gender differences, if they apply to cold-induced increases in *M*, are potentially of sufficient magnitude to warrant their consideration in a model of *ST* in cold stressed females.

3.1 Objectives

This report is a progress report which describes the results from the first of a series of studies carried out to address the issues raised above. Specifically this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The

objectives were: to determine the magnitude of $\dot{M}$ during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling $\dot{M}$; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on $\dot{M}$ and body temperature regulation during cold stress.

3.2 Hypotheses

a. The relative increase in $\dot{M}$ caused by cold water immersion will be less in females than what has previously been reported for males.

b. Compared to previous reports for males, a smaller proportion of the increase in $\dot{M}$ will be due to increases in carbohydrate oxidation.

c. Contrasting with what has been reported for males, muscle glycogen depletion of large skeletal muscle groups will not impair body temperature regulation during cold water immersion.

4. METHODS

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Martineau and Jacobs, 1988,1989a). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry, measurement of hormones and metabolites in venous blood, and measurement of metabolites in muscle biopsy samples.

Nineteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects did not donate blood for 30 days prior to or during participation in this study.

Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the Scientific Authority and medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving

medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body density by hydrostatic weighing. Maximum aerobic power ($\dot{V}O_2max$) was assessed separately for arm cranking exercise and for leg cycle ergometry so that appropriate exercise intensities for glycogen depletion exercise of both the upper and lower body could subsequently be determined. To familiarize the subjects with the laboratory setting and test procedures they were also immersed to the shoulders in 18°C water for 15 min.

4.1 Experimental design

On three subsequent visits the subjects were immersed in 18°C water, with identical procedures and measurements occurring for each cold exposure. The first immersion (control) was done after a 3-day period during which the subjects consumed an uncontrolled mixed diet. The two other experimental immersions followed 2.5 days of a specific dietary and/or exercise regimen (as described below) designed to elicit low or high glycogen levels in large skeletal muscle groups. The order of these dietary manipulations was counterbalanced among the subjects, with at least 6 days of uncontrolled mixed diet given between the two dietary regimes.

4.2 Standardization of menstrual and diurnal cycles

Initially, there was an attempt to have subjects undergo the various trials during the follicular phase of their menstrual cycle. However, the logistics of this timetable meant that some subjects would require two to three months to complete testing , which not only increased the likelihood of subject attrition but would also increase the risk of greater random trial-to-trial variation in measured responses. Moreover, Mittelman et al. (personal communication, 1997) recently showed that there was no effect of menstrual cycle phase on body temperature regulation during cold stress. We therefore felt that risk of significant random experimental error introduced by a long interval between immersions was greater than the risk that the menstrual cycle phases would confound our data

interpretation. Thus, we did not attempt to standardize the menstrual cycle phase for the various cold water immersions.

The subjects were always immersed at the same time of day to avoid possible diurnal effects. They were asked to abstain from alcohol for 48 hours before a trial, not exercise within 24 hours of a trial, and fast for 12-14 hours before each trial.

4.3 Glycogen depleting exercise

Three days before the two experimental immersions, two-legged exercise was performed for 60 min on a cycle ergometer at a power output requiring about 70% $\dot{V}O_2max$. This was followed by four or five 1-min exercise bouts performed at about 90% $\dot{V}O_2max$. This procedure has been demonstrated previously to result in depletion of glycogen from both main muscle fiber types of the leg muscles (Jacobs, 1981). After a 30-min rest period, the same exercise protocol was repeated using an arm-crank ergometer; the corresponding exercise intensities were about 60 and 90% arm $\dot{V}O_2max$.

4.4 Dietary manipulations

Immediately at the end of the exercise sessions, the subjects were instructed and informed about the composition of different types of food associated with carbohydrate (CHO)-reduced and CHO-rich diets. A list of suggested foods and a sample meal plan was provided to each subject. For 2.5 days the subjects consumed a free choice of foods within these guidelines. They were instructed verbally and with written guidelines that CHO should make up 90-95% of total nutritional consumption during the CHO-rich diet, but only 5-10% in the CHO-poor diet. The subjects were asked to weigh themselves daily, and to try to maintain the same total energy intake as usual throughout the diet treatment to ensure that body weight was being maintained. Previously, this laboratory used the same protocol to significantly alter the glycogen content in the vastus lateralis in males subjects (Martineau and Jacobs, 1989). Body weight was determined prior to each immersion.

4.5 Cold water immersion

On the day of each immersion the subjects reported to the lab in a 12-h post absorptive state, clad in a two-piece bathing suit. They inserted a rectal probe, were instrumented with 12 calibrated heat-flow transducers, bipolar ECG skin electrodes, and an intravenous catheter. They lay quietly in a supine position for 30 min at 23°C. Their resting metabolic rate was determined during the final 10 min of rest using a semi-automated metabolic cart system. Using an electrical winch system, they were lowered at a standardized rate into a water immersion calorimeter, with water temperature at 17.8°C, and remained in a supine position immersed to the neck in stirred chilled water until one of the following criteria, established before the experiment, was reached: 90 min elapsed, rectal temperature decreased to 35°C, or the subject asked to be withdrawn from the immersion tank.

4.6 Muscle biopsies

Muscle samples were taken from the right *quadriceps femoris vastus lateralis* just before water immersion (i.e. after the 30 minute rest period) and again from the same muscle just after each immersion, employing the percutaneous needle biopsy technique (Bergström, 1962). Skin and the underlying fascia were anaesthetized with 3 mL of xylocaine (2% epinephrine) after cleansing with an antiseptic solution (Betadine surgical scrub, Purdue Frederick Inc.). Both pre- and post immersion samples were taken from the same incision. Incisions were closed using Steri-Strip® (3M, St. Paul, MN). A water-proof dressing (Tegaderm®, 3M, St. Paul, MN) was placed over the Steri-Strip and then an elasticized bandage was wrapped around the thigh in an attempt to exert some pressure on the biopsy site and hopefully reduce the soreness that is frequently experienced in the thigh for 2-3 days after the biopsy. This bandage was left on the leg during the immersion, removed for the post-immersion biopsy and then dry Steri-Strips® and a dry elasticized bandage were placed on the leg after the experiment. Subjects were instructed to leave the elasticized bandage on the leg for 3-4 hours; they were instructed to leave the Steri-Strips® in place for 5 days.

During subsequent immersions, incisions were made on the same leg but at least 3 cm away from the previous incision.

On two occasions the Tegaderm® did not adhere well when the antiseptic residue around the biopsy site was not thoroughly removed with an alcohol swab prior to making the incision; when this was observed the subject's leg was quickly removed and elevated above the water line until a new bandage could be applied to cover the incision.

No complications, such as subsequent infection, resulted from the biopsies. Subjects did, however, report varying intensities of muscle soreness in the thigh, sometimes lasting as long as 4-5 days after the biopsy. The intensities ranged from no soreness at all to some subjects who were in extreme discomfort for 24 hours after the experiment. No subject requested or required follow-up medical referral. One subject did have a minor skin rash response to the Steri-Strip®. One subject reported loss of sensation in a 5 cm^2 skin area around the biopsy site, which is slowly resolving but is still evident almost one year after the experiment.

4.7 Blood sampling

The protocol called for venous blood samples to be obtained from an antecubital vein just before immersion (i.e. after the 30 min rest period) and after 5, 30, 60, and 90 min of immersion. Difficulties were encountered almost immediately in obtaining sufficient volume of blood, probably due to the combination of extreme vasoconstriction and decreased blood flow to the forearm. It is noteworthy that the incidence of problems in this regard was much higher than we have experienced when similar blood sampling methodologies were used with male subjects (Bourdon et al., 1995). Initially a 20 gauge, 1.0 inch catheter (Insyte™, Becton Dickinson) was used in conjunction with a slow infusion of warmed isotonic saline containing no heparin. Larger and longer catheters (20 gauge 8 inch and 18 gauge 1.25 inches) were also tried in an attempt to improve line patency, but without success. The best results were achieved using a heparin lock (10 U/mL) with the 20 gauge 1 inch catheter, and this system was used for the remainder of the experiment. A water-proof dressing

(Tegaderm®) was placed over the site where the catheter pierced the skin. During immersions the arm was supported just above the water by an adjustable sling hanging from the ceiling; this was done to reduce water contact with the catheter site and in an attempt to reduce local vasoconstriction. Ten mL blood samples were drawn and divided into 3 tubes which were kept on crushed ice: 2 mL were dispensed into heparin treated tubes for the subsequent determination of glucose, lactate, beta-hydroxy-butyrate, hematocrit and hemoglobin; 4 mL were expelled into a tube treated with EGTA (90 mg/mL) and glutathione (60 mg/mL), centrifuged and the plasma was frozen for subsequent determination of catecholamines; 4 mL were dispensed into a chilled, EDTA-treated tube (2 mL from this tube were in turn dispensed into a tube containing Trasylol® for subsequent determination of glucagon; the remaining 2 mL were centrifuged and aliquot of the plasma was subsequently used for the determination of free fatty acids, glycerol and insulin). All samples were stored at -20°C until frozen and then stored at -70°C until assayed.

4.8 Biochemistry

Hematocrit was determined by centrifugation (Autocrit Ultra3 centrifuge). Commercially available kits were used to measure concentrations of plasma glucagon (Glucagon RIA kit, Diagnostic Products Corporation, California), plasma insulin (Pharmacia Insulin RIA 100, Pharmacia, Uppsala, Sweden), and free fatty acids (WAKO™ NEFA kit, Texas). Glucose and hemoglobin were assayed using automated spectrophotometric techniques (Hemocue™). Plasma samples were analyzed for glycerol concentration after deproteinization (Boobis and Maughan, 1983), lactate and beta-hydroxy-butyrate (Maughan, 1982). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (Zamecnik, 1997). Changes in plasma volume were calculated from the changes in hematocrit and hemoglobin concentration (Dill and Costill, 1974).

Muscle tissue samples were freeze dried for at least 8 hours. Glycogen was assayed as glucose units following hydrochloric acid hydrolysis using a fluorometric enzymatic method (Karlsson, 1971).

To facilitate calculations of protein oxidation during the immersions, the subjects were asked to collect urine for 24 h beginning the morning of, and prior to, the immersion. The urine was subsequently assayed for its urea nitrogen concentration (Sigma Kit 640, Sigma Chemicals Co., MO, USA).

4.9 Temperature measurements

The immersion tank used is a whole body calorimeter with exterior dimensions of 224.5 cm length by 93.2 cm width and 77.6 cm high. The volume of water in the calorimeter for the immersions was about 1200 liters. The calorimeter was initially developed to control water temperature and determine heat loss from an immersed object, however, in this experiment, water temperature was not controlled. Instead, subjects were immersed at a water temperature of 17.8°C and water temperature was measured continuously during the immersion, and for at least 30 minutes before and after each immersion. The temperature of the water was measured at 10 different sites using calibrated thermistors (unsheathed Baxter Rectal Probes, 400 series) and the water was stirred continuously. Heat loss from the body to the calorimeter was calculated by comparing the rate of water temperature change during the immersion to that measured before and after the immersion. For reference purposes, here are some data for one of the subjects to exemplify the resolution of the calculations of heat loss to the calorimeter: rate of water temperature prior to immersion was 0.092°C/h; during the first minute of immersion it increased to 1.458°C/h; 4 min into the immersion the value decreased to about 50% of that peak value, and by the last few minutes of immersion the change in water temperature was only 0.15°C/h. The water's heat gain due to the loss of heat from the webbed stretcher on which the subject lay was quantified and subtracted from all calculations. An automated data acquisition system was used to record the water temperatures at a frequency of 12 samples/min.

During the immersions the following were measured continuously with an automated data acquisition system, and averaged each minute: rectal temperature (Pharmaseal® 400 Series, Baxter Healthcare Corporation, California), mean skin temperature and mean skin heat flow using a 12-point area-weighted system as described elsewhere (Vallerand et al., 1989). For measurement of skin temperature and heat flow, the same twelve heat flow sensors (Concept Engineering, model FR-025-TH44033-F8-F, Connecticut) were used throughout the entire experiment.

4.10 Respiratory gas exchange measurements

Respiratory gases were monitored using a semi-automated metabolic cart system during the 30 min rest period prior to immersion, and continuously throughout the immersion, with the exception of a 5 minute break for re-calibration purposes after 25 min of immersion. For this purpose the subject was connected to a mouth-piece, breathing valve, and hose, which directed the expired gases to a 5 liter mixing box, which was connected in series to a ventilation module which measured expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, California). A sample line directed gases from the mixing box to oxygen (AMETEK Model S-3A11, Applied Electrochemistry, Paoli, Pennsylvania) and carbon dioxide (AMETEK Model CD-3A, Applied Electrochemistry, Paoli, Pennsylvania) analyzers. Commercially available microcomputer based software (Vista/Turbofit Software, version 3.10, Vacumetrics Inc., Ventura, California) was used to register the data each minute, and to convert the values into STPD units of oxygen consumption and carbon dioxide production.

4.11 Calculation of metabolic heat production and substrate contributions

Metabolic heat production rates ($\dot{M}$) were calculated from the respiratory gas exchange measurements of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio (RER) according to Pérronet et al. (1991) Data collected during the first 5 min of immersion were not used in any related

calculations because of the reflex hyperventilation caused by cold water immersion.

The rates of carbohydrate and fat oxidation (CHO_{ox} and FAT_{ox}, respectively) were calculated using the non-protein oxygen consumption and the non-protein respiratory exchange ratio. Protein oxidation (PRO_{ox}) was assessed using the urinary urea nitrogen excretion rates (Vallerand et al., 1993). Detailed descriptions of the calculations for substrate oxidation rates are available in Vallerand et al. (1995).

4.12 Statistical analyses

Twelve subjects participated in the control immersion but only nine of them completed the other experimental immersions. The reasons for the subject attrition are described in the Results. In order to exploit as much data as possible the data were first analyzed to determine the effects of water immersion alone on the measured variables, without consideration of the dietary treatments. These data were analyzed using a one-factor analysis of variance for repeated measures. Of the twelve subjects completing the control immersions, only 10 completed 85 minutes of immersion and two subjects asked to be removed from the water after 60 min. Therefore, the control immersion data have been presented separately for two groups: a group (n=10) that completed 85 minutes of the control immersion (C85), and a group (n=12) that completed at least 60 min of immersion (C60).

The statistical analyses of the effects of the dietary treatments were then done using a data base of results from those subjects who completed all three experimental immersions (i.e. the control, high carbohydrate, and low carbohydrate immersions). These results were analyzed with a two-factor analysis of variance for repeated measures to determine the main effects of the immersion and the diet factors (Low, Control, or High), and any significant interactions between significant main effects. Of the nine subjects who completed all treatment immersions, one completed only 60 min of the control immersion. Therefore, these subjects were divided into two groups for the purpose of data

analysis and presentation. One group (n=9) completed at least 60 min of each trial (T60), and the other group (n=8) completed 85 min of every trial (T85). Statistical analysis was only performed on the groups that completed 85 min (C85 and T85). Unless otherwise noted, data are presented as mean values ± standard deviation. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

5. RESULTS

5.1 Subject attrition

Nineteen subjects signed consent forms and completed all familiarization and medical screening procedures. Of these, seven subjects dropped out of the experiment: two due to pregnancy, two due to scheduling conflicts, and three because of the discomfort they perceived associated with the cold water immersion and/or the invasive procedures.

All of the remaining twelve subjects completed the control immersion and tolerated at least 60 min (C60); ten of these 12 completed the full 90 min of the control immersion (C85). The other two subjects voluntarily terminated the immersion at 60 min, one due to cold discomfort and the other due to the need to urinate and her unwillingness to do so in the immersion tank

Nine of the 12 subjects who did the control immersion also completed the other two experimental immersions. Three dropped out of the study at various stages, one because of apprehension about the biopsy procedure, one for medical reasons, and one due to scheduling conflicts.

Much of the data based on the respiratory gas exchange analysis (e.g. metabolic rate and substrate oxidation rates) are reported for an immersion duration of 85 min because the mouthpiece was removed from the subject at that time, thus the use of the terms C85 and T85 to denote subjects who lasted the full 90 min of immersion in the control and treatment trials, respectively.

The duration of each immersion for each subject is presented in **Table 1.**

5.2 Subject characteristics

The physical characteristics of the subjects are presented in **Table 2**. Subjects had a mean age of 24 y and were of average height and weight. The mean relative body fat mass was normal (23%), however, it ranged widely, from 14% in a competitive distance runner to 35%. With reference to $\dot{V}O_2max$, the subjects were of average fitness although two subjects had superior fitness levels due to their involvement in competitive sport. Only two subjects took oral contraceptives. As stated earlier the phase of the menstrual cycle on the day of each immersion was not standardized, but it was recorded and this information is presented in **Table 3.**

5.3 Rectal temperature and rate of body heat loss

Control immersion. **Figure 1** displays the change in rectal temperature for each subject during the control immersion, and **Figure 2** shows the mean response. Rectal temperature decreased and rate of body heat loss increased significantly during immersion. There was a wide range of response, with the change in rectal temperature being significantly correlated with the % body fat content (r=0.82). **Figure 3** depicts the calculated rate of heat loss to the calorimeter; as intuitively expected, maximal heat loss rates occurred during the first few minutes of immersion; by 30 minutes into the immersion vasoconstriction was probably close to maximal and the rates of heat loss were only about 25% of the initial rates and slowly decreased to even lower rates during the remaining portion of the immersion.

Dietary treatment immersions. There was a significant decrease in rectal temperature during all immersions but no difference among treatment trials (**Table 4**). **Figure 4** shows that there was a tendency for rectal temperature to reach a higher peak value during the high carbohydrate trial, and for rectal temperature to be somewhat lower during the latter phases of the immersion, but these trends were not statistically significant. Similarly there was no difference among trials for the rate of heat loss (**Figure 5**).

5.4 Metabolic heat production

Control immersion. **Table 5** shows the values for $\dot{M}$ both before and during the immersion. **Figure 6** shows the mean values for all subjects and demonstrates that $\dot{M}$ increased significantly about 2.5-fold during the first 10 min of immersion, and then continued to increase progressively but much more slowly, such that the final values were about 3.1 times the pre-immersion values. For the C85 group, the $\dot{M}$ data were grouped into 30 min intervals to facilitate statistical comparisons; the average $\dot{M}$ during the first 30 min of immersion was not different from the mean value for the data collected from 30-60 min of immersion. Both of these first two intervals were significantly lower than the $\dot{M}$ measured during the last 30 min of immersion (**Figure 7**). The peak metabolic rate recorded for each subject during the immersion was expressed relative to that subject's $\dot{V}O_2max$ during exhaustive exercise (**Figure 8**). There was no relationship between the $\dot{V}O_2max$ values and the $\%\dot{V}O_2max$ elicited during shivering. During this immersion the percentages of $\dot{M}$ that were calculated to be derived from CHO_{ox}, FAT_{ox} and PRO_{ox}, were 36±11%, 59±12%, and 5±3%, respectively. Both CHO_{ox} and FAT_{ox} increased significantly during the water immersion compared to the pre-immersion values, while there was no change in PRO_{ox}.

Dietary treatment immersions. $\dot{M}$ did not differ significantly when the various trials were compared (**Table 6, Figure 9**); the mean±SD values for $\dot{M}$ during the immersions were 123±30, 124±37, and 112±36 W/m^2 for the control, high carbohydrate, and low carbohydrate trials, respectively. CHO_{ox} and FAT_{ox}, as a percent of the total $\dot{M}$, differed significantly across trials; % $\dot{M}$ due to CHO_{ox} was significantly less both at rest and during immersion for the low carbohydrate trial than the other two trials, and % $\dot{M}$ due to FAT_{ox} was significantly greater at rest and during immersion for the low carbohydrate trial than the other two trials (**Figure 10**). Individual values for the $\%\dot{M}$ attributed to carbohydrate, fat and protein oxidation are shown in **Table 7**.

5.5 Muscle glycogen concentrations

Control immersions. Both pre and post immersion muscle biopsy samples were successfully done on 11 of the 12 subjects. The remaining subject did not have complete data because she complained of discomfort during the biopsy procedure; it was decided not to proceed with the biopsy rather than cause the subject more discomfort. Muscle glycogen levels were significantly lower after immersion in C85, the net difference amounting to a change of about 20% of the initial glycogen levels. **Table 8** shows that each of the subjects, with one exception, had a lower glycogen level after immersion when compared to the pre-immersion concentration. The subject who showed a net increase in glycogen was in the C60 group, i.e. she only remained in the water for 60 min during the control immersion. The mean magnitude of the change was similar in the C60 and C85 groups, which suggests that the rate of glycogen utilization may have been greater earlier in the immersion and that much slower glycogen utilization was occurring later in the immersion.

Dietary treatment immersions. In contrast with expectations, the exercise/nutritional treatments did not significantly change the pre-immersion muscle glycogen levels; they were similar on all trials. Mean glycogen concentrations (in mmol glucose units/kg dry muscle) prior to the Control, High and Low trials were 489±128, 573±147, and 460±74, respectively. The ANOVA resulted in a significant main effect of time, without any interaction with treatment. Thus, muscle glycogen decreased significantly as a result of immersion, but the dietary treatments did not affect the magnitude of that decrease (**Figure 11, Table 9**). The glycogen utilization rate was determined for group T85 by dividing the pre-post difference in glycogen concentration by 85 minutes, although it is acknowledged that the rate of change is probably not linearly related with elapsed time; this rate of change was also similar across trials with mean values of 1.09, 0.66 and 0.80 mmol glucose units/kg dry muscle/min during control, high carbohydrate and low carbohydrate trials, respectively.

5.6 Blood metabolites and hormones

As described in a previous section, difficulties were encountered in obtaining blood samples during immersion in several subjects. In light of the number of missing data at varying time intervals, statistical analysis was limited to comparing the pre with the post immersion values, and only for the five to six subjects for whom both samples were available.

Control immersions. With the exception of beta-hydroxy-butyrate and epinephrine, which did not change, there was a significant increase in all other metabolite and hormone concentrations. Glucose increased from 4.6±0.17 to 5.23±0.25 mmol/L. Insulin increased from 7.9±.96 to 10.7±1.3 μU/mL, while glucagon also increased slightly, but significantly, from 97±8 to 109±11 pg/mL. FFA and glycerol concentrations increased from 0.40±0.07 to 0.82±0.09 mmol/L, and from 0.057±0.007 to 0.17±0.020 mmol/L, respectively. Lactate increased from 0.9±0.2 to 2.1±0.4 mmol/L. The mean values for epinephrine were 39±19 before immersion and 121±73 ng/mL after immersion, a difference which just failed to reach statistical significance for the five subjects for whom both pre and post immersion samples were available ($p=0.08$). The corresponding values for norepinephrine were 229±30 and 1192±212 pg/mL, which was a significant difference.

These changes in blood metabolite and hormone concentrations should be considered in light of the hemoconcentration which was reflected in a 18% decrease in plasma volume, although some of the changes are too great to be attributed only to the hemoconcentration, e.g. glycerol (205%), insulin (35.4%), FFA (102%) and lactate (125%).

Dietary treatment immersions. The low carbohydrate treatment resulted in significantly higher pre-immersion concentrations for FFA, glycerol, and beta-hydroxy-butyrate, when compared to the pre-immersion values for the control and high carbohydrate trials. The exercise/dietary treatments did not significantly change the pre-immersion concentrations of glucose, lactate, insulin, glucagon, epinephrine or norepinephrine. The changes of the various metabolites and hormones were similar on all trials, with significant increases occurring in

concentrations of glucose, lactate, insulin, FFA, glycerol, beta-hydroxy-butyrate, epinephrine and norepinephrine but no significant change in glucagon. The magnitude of the increase in concentration should once again be considered in light of the significant decrease in plasma volume which occurred on all trials, to a similar degree: -15% during the high carbohydrate trial, -16 % during the low carbohydrate trial, and -20% during the control trial (**Table 10**).

The individual data for the various blood measurements are shown in **Tables 11-19**.

6. DISCUSSION

This document is a progress report describing the results of the first in a series of projects designed to investigate whether gender differences in physiological responses to cold stress are of a sufficient magnitude to have implications for predictive models of human body temperature regulation. The purpose of this particular project was to "...clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of $\dot{M}$ during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling $\dot{M}$; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on $\dot{M}$ and body temperature regulation during cold stress."

Detailed analysis, interpretation of the results, and the implications for predictive modelling will be reserved for the Final Report. Some commentary follows below, however, regarding the testing of the specific experimental hypotheses for this particular project.

6.1 Hypothesis A:

"The relative increase in $\dot{M}$ caused by cold water immersion will be less in females than what has previously been reported for males."

As noted in the results, metabolic rate increased during the control immersions to values that were about 3.2 times resting metabolic rate (RMR), corresponding to about 35% of the peak oxygen uptake during exhaustive exercise. Males who were exposed to the same water temperature achieved a metabolic rate that was only slightly higher, about 3.5 times their RMR, corresponding to about 30% of their peak oxygen uptake during exhaustive exercise (Martineau and Jacobs, 1988). Given the fact that the females in the present investigation had a relative body fat content that was at least double that of the males, and that the females were therefore relatively more insulated than the males used by Martineau and Jacobs (1988), the current study suggests that shivering induces relative increases in metabolic rate in females that are very similar to what has been reported for males. Thus this hypothesis is rejected.

When, however, the data are expressed relative to body surface area, the male subjects of Martineau and Jacobs (1988) generated heat from shivering at rates that were about 20% higher than the females. Perhaps, this is not too surprising given the likelihood that a unit of male body surface area covers proportionately more underlying skeletal muscle than is the case for females.

Skeletal muscle is the prime source of the increased heat production during shivering thus the absolute magnitude of heat production will of course be significantly higher in males than females. The implications for survival time in the cold may be more a function of the heat production per unit lean body mass than per unit of body surface area. Thus these data will be re-visited with more sophisticated statistical techniques in our final report, when attempts will be made to compare the heat production rates between genders on the basis of the relative activation of skeletal muscle used during shivering. Such comparisons will require the completion of our final study which employs electromyography.

6.2 Hypothesis B:

"Compared to previous reports for males, a smaller proportion of the increase in $\dot{M}$ will be due to increases in carbohydrate oxidation."

This hypothesis was based on reports about energy substrate utilization during exercise. Specifically, it has been documented that during exercise of similar relative intensities women oxidize more lipids, and therefore decrease carbohydrate and protein oxidation, compared with men (Tarnopolsky et al., 1990, 1995; Phillips et al., 1993). These results were observed in exercise intensities eliciting 65% and 85% of peak oxygen uptake.

Using a similar cold water immersion protocol with males the $\%\dot{M}$ attributed to carbohydrate oxidation was calculated to be 45-50% (Martineau and Jacobs, 1989a,b). In the current investigation with female subjects, it was calculated that the contribution of carbohydrate oxidation amounted to 38-40% of $\dot{M}$. It should be noted that muscle glycogen levels were significantly lower after immersion than before immersion for all trials, suggesting that muscle glycogen contributes to total carbohydrate oxidation during shivering in females, as was reported previously for male subjects.

The actual quantitative difference in heat production which would be associated with this difference in the relative contribution of carbohydrates to total energy expenditure must be extrapolated to longer duration cold water immersions in order to evaluate the implications for the prediction of survival times. This issue will be addressed in our Final Report. In the interim, this hypothesis is accepted.

6.3 Hypothesis C:

"Contrasting with what has been reported for males, muscle glycogen depletion of large skeletal muscle groups will not impair body temperature regulation during cold water immersion."

The results from the current investigation did not enable us to address this hypothesis because we were not successful in causing the subjects to deplete their muscle glycogen concentrations to levels comparable with what we previously

induced in male subjects. In spite of having attempted to use a protocol for glycogen depletion that was identical to that used by Martineau and Jacobs (1989a), muscle glycogen concentrations after the low carbohydrate dietary treatment were not significantly different than those on the other two trials. Interestingly, the high carbohydrate dietary treatment also did not result in the expected effect of increasing muscle glycogen levels. Since we depended on the subjects to follow our dietary guidelines, it is possible that their adherence was not satisfactory. However, this is not the first time that gender differences have been reported in attempts to manipulate muscle glycogen levels. Tarnopolsky et al. (1995) reported that in contrast to what was observed in male subjects who were exposed to an identical protocol, the females did not increase muscle glycogen levels with a protocol that has repeatedly been reported to cause quite dramatic increases in male subjects.

The dietary treatment in the current study did, however, have measurable effects on indices of energy metabolism both at rest and during the immersion. Specifically, the contribution of fat oxidation to total metabolic heat production was higher, and carbohydrate oxidation was lower, both at rest and during the immersion after the low carbohydrate dietary treatment when compared to the other two immersions. The absolute $\dot{M}$ was not affected, however, nor was the rate of change in rectal temperature different across trials.

7. SUMMARY AND CONCLUSIONS

A. Data collection for the first phase of the study was completed as scheduled. There were a couple of difficulties encountered which are noteworthy. The protocol used was a duplicate of one used previously with male subjects. It appeared to be much more arduous for the female subjects, resulting in significantly more subject attrition. Attrition was compensated by recruiting more subjects, so that we were successful in collecting data for the number of subjects originally projected. Vasoconstriction and lack of blood flow to the extremities resulted in more difficulty than expected in obtaining venous blood samples during the cold water immersion.

B. Shivering induced via cold water immersion results in relative increases in metabolic rate which are similar to those previously observed in male subjects. The absolute metabolic heat production for females will be significantly less than for the average male because of differences in body size; the implications in terms of body temperature regulation during more prolonged cold stress, will be analyzed with more sophisticated mathematical modelling techniques in our final report. Future analyses will consider the gender-related differences in body size and relative lean body mass.

C. Females used muscle glycogen as one of the carbohydrate energy stores to fuel shivering, as reported previously for male subjects. This investigation was not successful in addressing the issue of whether manipulations of glycogen levels prior to immersion would affect subsequent thermoregulation during cold stress. A protocol which has traditionally resulted in the ability to significantly manipulate muscle glycogen stores in male subjects, was ineffective when applied to the female subjects in this investigation. There is only one prospectively designed study which compared the responses of males and females to classical "glycogen loading" protocols, and it reported that females did not increase their muscle glycogen levels. Our investigation suggests that females may rapidly replete muscle glycogen stores after depletion, even when nutritional carbohydrate intake is very low.

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Table 1. Duration of immersion for all subjects during the control and immersion and during immersions after the low carbohydrate (CHO) and high CHO diets.

Subject	Time (min)		
	Control	High CHO	Low CHO
AA	90	90	90
TB	90	90	90
MD	90	90	90
HH	90	90	90
DL	90	90	90
GM	60	90	90
JN	90	90	90
MP	90	90	90
CS	90	90	90
AG	90		
WH	90		
KK	60		

Table 2. Physical characteristics of subjects completing all three trials (T60), 85 min of all three trials (T85), only the control trial (C60) and 85 min of the control trial (C85).

Subject	HEIGHT (cm)	WEIGHT (kg)	AGE (yrs)	BSA (m^2)	BODY FAT (%)	Lean Mass (kg)	VO_2 max - arms (l/min)	VO_2 max - arms (ml/kg/min)	VO_2 max - arms (ml/kgLBM/min)	VO_2 max - legs (l/min)	VO_2 max - legs (ml/kg/min)	VO_2 max - legs (ml/kgLBM/min)	Oral Contraceptive
AA	178.8	70.2	20	1.874	21.4	55.18	1.64	22.8	29.72	2.71	37.9	49.11	Yes
TB	167.5	61.6	18	1.694	26.1	45.5	2.19	34.9	48.13	2.87	45.8	63.08	No
MD	160	59.4	30	1.616	27.1	43.3	1.08	16.6	23.28	1.73	28.3	39.95	No
HH	175.5	80.8	23	1.975	35	52.5	2.28	27.8	43.43	3.14	38.3	59.81	No
DL	170	61.7	37	1.709	14.3	52.88	1.93	30.6	36.50	3.13	49.9	59.19	No
GM	165.1	63.5	33	1.69	16.9	52.77	1.88	29.7	35.63	2.65	43	50.22	No
JN	151.5	63.3	19	1.588	32.3	42.85	1.3	20.3	30.34	2.04	32	47.61	No
MP	165	58.2	19	1.613	25.3	43.48	1.4	24.9	32.20	1.9	33.5	43.70	No
CS	169	65.3	22	1.719	19.6	52.5	1.8	26.6	34.29	2.6	39.2	49.52	No
mean, T60 (n=9)	166.93	64.89	24.56	1.72	24.22	49.00	1.72	26.02	34.83	2.53	38.66	51.35	
± SD	8.07	6.91	6.98	0.13	6.85	5.06	0.40	5.59	7.43	0.52	6.85	7.77	
mean, T85 (n=8)	167.16	65.06	23.50	1.72	25.14	48.52	1.70	25.56	34.74	2.52	38.11	51.50	
± SD	8.60	7.36	6.65	0.14	6.71	5.20	0.43	5.79	7.93	0.56	7.11	8.29	
AG	174	70.8	24	1.845	16.2	59.33	1.51	28.7	25.45	2.6	50.2	43.82	Yes
WH	167	70.9	23	1.792	21.6	55.59	1.58	22.3	28.42	1.8	25.4	32.38	No
KK	150.5	52.3	19	1.46	22.7	40.43	2.09	29.4	51.69	3.08	43.2	76.18	No
mean, C60 (n=12	166.16	64.83	23.92	1.71	23.21	49.69	1.72	26.22	34.92	2.52	38.89	51.21	
± SD	8.71	7.46	6.17	0.14	6.30	6.20	0.37	5.06	8.81	0.52	8.00	11.74	
mean, C85 (n=10	167.83	66.22	23.50	1.74	23.89	50.31	1.67	25.55	33.18	2.45	38.05	48.82	
± SD	9.13	7.81	5.74	0.15	6.27	6.42	0.38	5.18	9.24	0.55	8.28	12.31	

Table 3. Day of menstrual cycle during immersions.

Subject	Day of Cycle During Immersion		
	Control	High CHO	Low CHO
AA	36	32	25
TB	3	20	8
MD	20	22	28
HH	23	26	5
DL	24	20	3
GM	7	19	12
JN	90+	90+	90+
MP		6	2
CS	22	17	10
AG	2		
WH	9		
KK	21		

Table 4. Mean rectal temperature during all three immersion trials.

Time (min)	Tre (°C) in group T60						Tre (°C) in group T85					
	CONTROL		HIGH CHO		LOW CHO		CONTROL		HIGH CHO		LOW CHO	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Pre	37.00	0.18	36.98	0.26	37.03	0.23	37.01	0.2	36.98	0.28	37.05	0.24
10	37.15	0.23	37.13	0.27	37.09	0.30	37.14	0.24	37.13	0.29	37.12	0.30
20	37.15	0.25	37.20	0.28	37.10	0.32	37.16	0.26	37.21	0.29	37.14	0.32
30	37.09	0.37	37.20	0.30	37.06	0.37	37.11	0.39	37.24	0.30	37.11	0.38
40	37.00	0.43	37.15	0.30	36.97	0.43	37.01	0.46	37.20	0.28	37.01	0.44
50	36.88	0.50	37.03	0.32	36.80	0.48	36.89	0.54	37.08	0.29	36.85	0.48
60	36.73	0.57	36.89	0.31	36.61	0.54	36.75	0.61	36.94	0.29	36.68	0.53
70							36.64	0.61	36.79	0.29	36.55	0.58
80							36.53	0.63	36.68	0.31	36.44	0.60
85							36.48	0.65	36.48	0.43	36.41	0.59

Table 5. Mean metabolic rate responses during the control immersion.

Time (min)	*M* ($W \cdot m^{-2}$) in C60		*M* ($W \cdot m^{-2}$) in C85	
	Mean	± SD	Mean	± SD
Pre	43.24	4.22	43.48	4.45
10	108.34	22.22	106.52	23.90
20	120.36	42.06	107.12	31.41
30	129.09	38.90	118.52	31.62
40	129.82	37.07	120.22	31.29
50	129.72	34.17	123.87	33.42
60	131.67	27.51	128.60	27.97
70			138.45	25.17
80			137.32	31.82
90			140.10	26.52

Table 6. Mean metabolic rate responses during all three immersion trials.

Time (min)	*M* ($W \cdot m^{-2}$) in group T60						*M* ($W \cdot m^{-2}$) in group T85					
	CONTROL		HIGH CHO		LOW CHO		CONTROL		HIGH CHO		LOW CHO	
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Pre	43.67	4.34	43.09	6.07	41.17	2.71	44.20	4.31	42.51	6.22	40.79	2.63
10	108.92	24.01	119.76	38.99	94.42	35.47	108.70	25.65	113.62	36.74	92.78	37.55
20	114.03	39.75	127.62	49.04	110.21	38.87	105.63	32.86	117.74	41.76	105.36	38.53
30	127.70	42.09	131.42	53.82	116.23	40.44	118.22	33.14	118.91	41.24	110.64	39.33
40	128.34	40.06	135.83	60.18	113.30	42.15	119.69	32.62	120.63	41.99	107.24	40.65
50	126.84	37.12	128.42	56.17	110.79	38.14	120.43	33.94	113.55	36.48	103.95	34.37
60	129.53	30.37	134.34	52.07	122.55	35.90	124.98	29.00	121.04	35.75	116.60	33.30
70							134.79	23.93	130.96	37.78	123.77	35.72
80							130.54	27.50	133.23	35.62	121.58	33.84
85							139.25	28.12	141.80	29.98	124.49	31.18

Table 7. The relative proportion of metabolic heat production (%M) atttributed to carbohydrate, fat, and protein oxidation.

	Carbohydrate oxidation (%M)			Fat oxidation (%M)			Protein oxidation (%M)		
Subjects	Control	High	Low	Control	High	Low	Control	High	Low
GM	51.60	76.78	60.61	44.67	20.90	33.42	3.72	2.33	6.06
HH	35.09	32.17	15.16	54.87	57.23	76.13	10.03	10.60	8.71
MD	30.88	41.89	27.79	66.82	55.37	68.05	2.30	2.74	4.16
TB	43.78	42.56	12.89	49.63	52.89	79.18	6.59	4.54	7.93
MP	36.42	31.77	22.15	58.72	66.38	73.08	4.86	1.86	4.77
AA	24.15	28.49	24.74	71.44	67.02	68.56	4.42	4.49	6.70
DL	58.17	32.45	29.93	38.39	64.69	66.04	3.44	2.85	4.03
JN	47.34	48.34	48.46	43.25	41.96	39.57	9.41	9.70	11.97
CS	26.03	36.01	11.29	68.15	59.65	82.40	5.82	4.34	6.31
Mean, T60	39.27	41.16	28.11	55.10	54.01	65.16	5.62	4.83	6.74
±SD	11.70	14.81	16.59	11.97	14.68	17.18	2.65	3.18	2.53
Mean, T85	37.73	36.71	24.05	56.41	58.15	69.13	5.86	5.14	6.82
±SD	11.49	6.84	12.04	12.09	8.37	13.24	2.73	3.25	2.69

Table 8. Muscle glycogen concentration (mmol glucose/kg dry muscle) pre and post control trial cold water immersion.
* indicates significant difference from pre ($p<0.05$)

	Control	
Subject	Pre	Post
HH	555.8	397.78
AA	500.38	400.58
TB	436.58	418.94
MD	285.06	274.27
GM	329.6	346.57
CS	705.33	510.1
JN	564.71	524.77
MP	474.41	435
DL	552.51	327.22
AG	414.85	342.8
KK	484.3	475.18
Mean,(C60)	482.14	404.84
± SD	116.67	78.68
Mean, (C85)	498.85	403.5*
± SD	117.44	81.81

Table 9. Muscle glycogen concentration (mmol glucose•kg^{-1} dry muscle) during three cold water immersion trials.
* indicates significant difference from pre for the specific immersion ($p<0.05$).

	Control		Low CHO		High CHO	
Subjects	Pre	Post	Pre	Post	Pre	Post
HH	555.8	397.78	322.58	257.84	521.26	464.76
AA	500.38	400.58	543.77	462.48	500.02	442.63
TB	436.58	418.94	500.72	464.8	903.09	756.67
MD	285.06	274.27	479.22	432.49	474.13	414.21
GM	329.6	346.57	440.85	262.79	580.61	500.77
CS	705.33	510.1	425.45	327.88	493.99	393.42
JN	564.71	524.77	569.88	415.48	718.12	674.51
MP	474.41	435	416.58	360.37	445.09	329.04
DL	552.51	327.22	440.1	403.25	412.95	519.15
Mean, T60	489.38	403.91	459.91	376.38	561.03	499.46
± SD	128.02	81.47	74.03	79.21	156.04	136.55
Mean, T85	509.35	411.08*	462.29	390.57*	558.58	499.3*
± SD	120.95	84.01	78.77	71.39	166.63	145.98

Table 10. Change in blood plasma volume during cold water immersions.

	Blood Plasma Volume (% change from rest)											
	Time (min) during Control Immersion				Time (min) during High CHO immersion				Time (min) during Low CHO immersion			
SUBJECT	5	30	60	90	5	30	60	90	5	30	60	90
GM									-8.51	-12.58	-15.12	-18.86
TB					-5.65	-6.28	-13.1	-11.37	-5.16	-12.71	-9.59	-18.91
HH	-9.87	-12.19	-12.76	-11.28	-9.32	-12.61	-9.63	-14.66	-8.95	-10.37	-8.5	-11.89
MD	-15.66	-12.14	-16.49	-21.99	-8.77	-11.64	-11.66	-17.53	-6.66	-7.26	-6.66	-18.29
AA	-8.8	-17.2	-21.28	-23.17		-13.26		-21.54	-12.93	-16.47	-19.24	-25.22
JN	-11.62	-14.99	-21.26	-36.13					-9.01	-12.46	-17.75	
DL		-13.57	-18.01	-19.5			-17.67	-24.17	-7.03	-11.49	-15.18	-20.27
MP					-1.79		-19.74					
CS	-8.02	-12.15		-20.42	-6.97	-8.66	-11.17	-19.5	-1.3	-10.76	-12.36	-15.94
n	5	6	5	6	5	5	6	6	8	8	8	7
mean	-10.79	-13.71	-17.96	-22.08	-6.50	-10.49	-13.83	-18.13	-7.44	-11.76	-13.05	-18.48
± SE	0.61	0.34	0.71	1.34	0.60	0.59	0.67	0.77	0.42	0.33	0.57	0.58

Table 11. Changes in blood glucose levels before and after cold water immersions.

	GLUCOSE (mmol/l)					
	CONTROL		HIGH CHO		LOW CHO	
Subject	PRE	POST	PRE	POST	PRE	POST
GM	4.40	•	4.15	•	3.90	4.95
TB	4.85	•	4.55	5.40	4.45	5.15
HH	4.45	4.70	4.25	4.55	4.95	5.50
MD	3.85	4.60	4.25	4.55	4.25	4.70
AA	4.70	6.30	4.30	5.60	4.60	5.05
JN	4.85	5.20	4.90	•	4.80	•
DL	5.00	5.50	4.70	5.40	4.60	6.20
MP	•	•	4.60	•	4.80	•
CS	4.60	5.10	4.60	5.40	4.50	5.80

Table 12. Changes in blood lactate levels before and after cold water immersions.

	LACTATE ($mM \cdot L^{-1}$)					
	CONTROL		HIGH CHO		LOW CHO	
SUBJECT	PRE	POST	PRE	POST	PRE	POST
GM	0.36	•	1.24	•	0.44	2.75
TB	0.56	•	0.99	1.11	0.68	1.65
HH	1.01	0.83	0.99	1.55	0.63	1.05
MD	0.58	1.77	0.86	0.80	0.74	1.67
AA	0.47	1.44	0.49	2.90	0.61	2.65
JN	1.70	1.91	1.08	•	1.09	•
DL	1.11	3.41	0.44	3.66	0.33	4.15
MP	•	•	0.42	•	•	•
CS	0.66	3.08	1.24	1.75	0.68	1.93

Table 13. Changes in blood insulin levels before and after cold water immersions.

	INSULIN ($uU \cdot ml^{-1}$)					
	CONTROL		HIGH CHO		LOW CHO	
SUBJECT	PRE	POST	PRE	POST	PRE	POST
GM	3.50	•	5.41	•	4.20	7.18
TB	8.17	•	9.03	9.98	4.32	4.59
HH	7.65	9.28	7.65	8.91	7.87	8.06
MD	5.70	6.21	6.56	7.55	6.72	6.33
AA	5.21	8.77	5.26	9.08	5.92	9.54
JN	10.59	12.44	7.14	•	11.12	•
DL	7.44	14.12	3.00	8.84	3.93	10.97
MP	•	•	7.23	•	•	•
CS	10.70	13.30	6.23	11.07	5.13	8.43

Table 14. Changes in blood glucagon levels before and after cold water immersions.

	GLUCAGON ($pg \cdot ml^{-1}$)					
	CONTROL		HIGH CHO		LOW CHO	
	PRE	POST	PRE	POST	PRE	POST
GM	74.03	•	82.99	•	80.76	97.38
TB	68.05	•	81.77	87.57	85.93	78.31
HH	98.18	114.67	90.15	96.79	96.41	97.76
MD	73.79	79.02	73.67	97.3	103.55	90.91
AA	96.24	98.69	90.08	106.94	100.44	104.45
JN	93.96	108.96	121.22	•	109.35	•
DL	120.81	145.54	101.79	146.98	116.44	146.05
MP	•	•	83.98	•	•	•
CS	122.18	•	100.38	115.95	108.06	128.42

Table 15. Changes in blood free fatty acid levels before and after cold water immersions.

	FFA ($mmol \cdot l^{-1}$)					
	CONTROL		HIGH CHO		LOW CHO	
Subject	PRE	POST	PRE	POST	PRE	POST
GM	0.49	•	0.10	•	0.70	1.22
TB	0.45	•	0.41	0.76	0.76	1.17
HH	0.45	0.63	0.33	0.57	0.35	0.61
MD	0.55	1.06	0.35	0.83	0.57	0.94
AA	0.44	1.00	0.34	0.77	0.59	1.00
JN	0.36	0.65	0.29	•	0.41	•
DL	0.13	0.76	0.43	1.25	0.44	1.41
MP	•	•	0.66	•	•	•
CS	0.51	•	0.41	0.69	0.80	1.17

Table16 . Changes in blood glycerol levels before and after cold water immersions.

	GLYCEROL ($mmol \cdot l^{-1}$)					
	CONTROL		HIGH CHO		LOW CHO	
SUBJECT	PRE	POST	PRE	POST	PRE	POST
GM	0.039	•	0.055	•	0.081	0.235
TB	0.044	•	0.040	0.121	0.045	0.150
HH	0.070	0.096	0.047	0.094	0.048	0.092
MD	0.051	0.194	0.046	0.119	0.058	0.167
AA	0.040	0.219	0.042	0.188	0.089	0.209
JN	0.085	0.135	0.076	•	0.094	•
DL	0.053	0.219	0.064	0.272	0.059	0.313
MP	•	•	0.074	•	•	•
CS	0.043	0.179	0.053	0.169	0.092	0.263

Table 17. Changes in beta-hydroxybutyrate levels before and after cold water immersions.

	ß-HB ($mmol \cdot l^{-1}$)					
	CONTROL		HIGH CHO		LOW CHO	
Subject	PRE	POST	PRE	POST	PRE	POST
GM	0.17	•	0.08	•	0.44	0.70
TB	0.14	•	0.07	0.06	1.62	1.17
HH	0.10	0.11	0.09	0.09	0.10	0.18
MD	0.40	0.67	0.14	0.29	0.60	0.57
AA	0.22	0.45	0.13	0.41	0.48	0.73
JN	0.09	0.08	0.11	•	0.09	•
DL	0.08	0.11	0.31	0.60	0.24	0.50
MP	•	•	0.41	•	•	•
CS	0.28	0.40	0.09	0.13	0.56	0.77

Table 18. Epinephrine levels ($ng \cdot ml^{-1}$) during three immersion trials.

	Control					High CHO					Low CHO				
Subject	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
AA	40	118	113	46	189	28		111		147	59	204	193	139	132
GM	40					28					42	132	190	145	119
TB	217					87	67	113	115	29	67	67	92	27	41
HH	65	71	55	42	49	30	80	84	51	50	27	60	43	36	64
MD	47	11	95	98	169	16	34	64	51	19	24	66	56	51	49
JN	26	60			35	19					13				
DL	17		182	119	161	56			121	148	14	180	130		225
MP						24									
CS	18	72	91			19	82	59	48	34	21		69	56	59

Table 19. Norepinephrine levels ($pg \cdot ml^{-1}$) during three immersion trials.

	Control					High CHO					Low CHO				
Subject	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
AA	194	642	974	758	1097	196		1053		900	151	670	732	989	1002
GM	369					747					441	1060	1858	2112	1923
TB	175					316	1272	2060	2704	2808	267	1524	2418	2954	2531
HH	209	678	599	814	925	265	622	556	832	1281	280	767	900	1015	872
MD	224	559	723	1007	1393	190	390	387	549	457	266	560	634	857	1642
JN	252	837			1122	192					233				
DL	268		1291	1355	1423	250			1398	1221	193		958	1453	1337
MP						330									
CS	228	799	963			248	755	906	1209	1345	263		730	1304	1737

10. FIGURES

Figure 1. Individual rectal temperature response to cold water immersion.

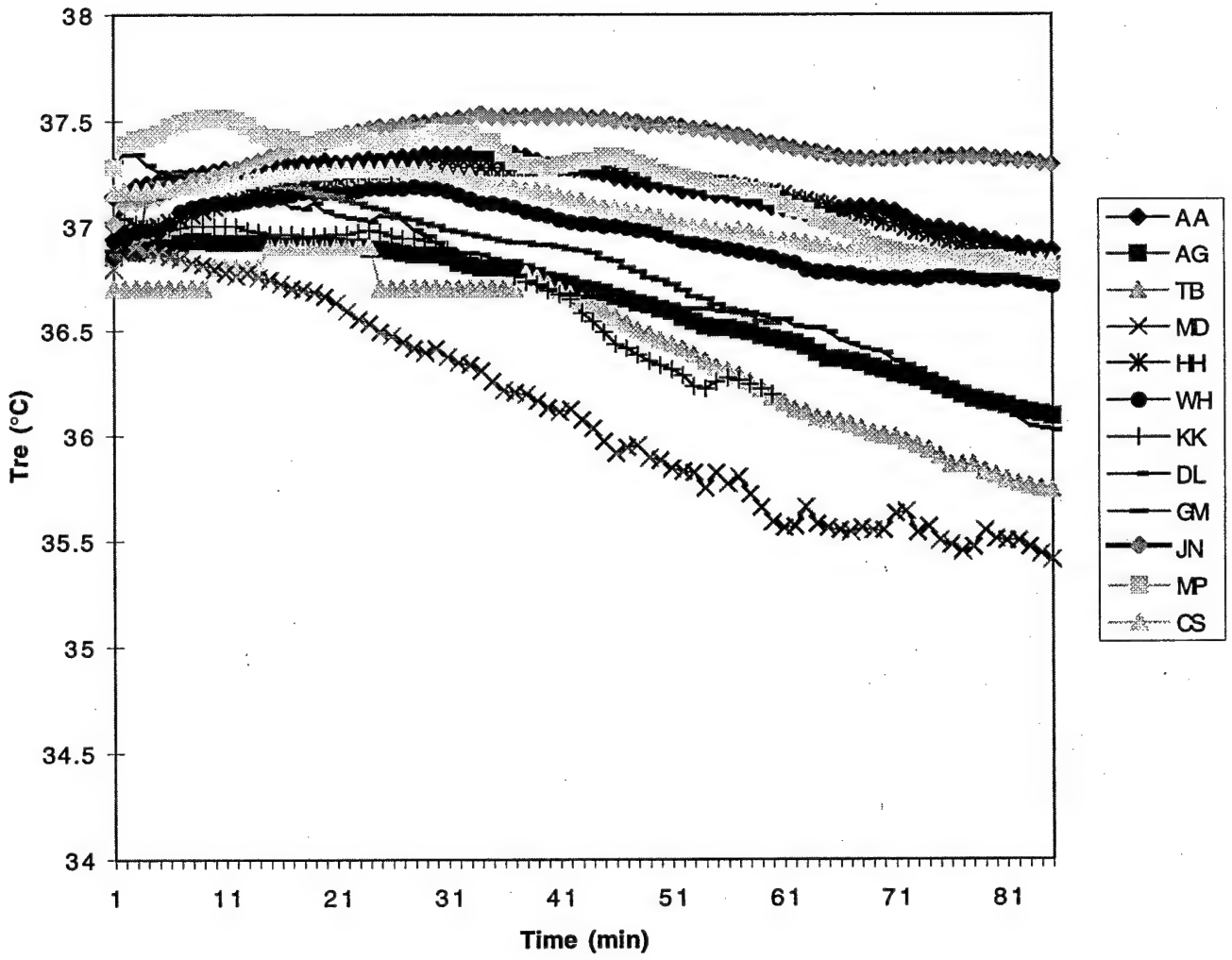

Figure 2. Rectal temperature changes during control trial cold water immersion for subjects who endured 85 min (C85) or 60 min (C60) of immersion.

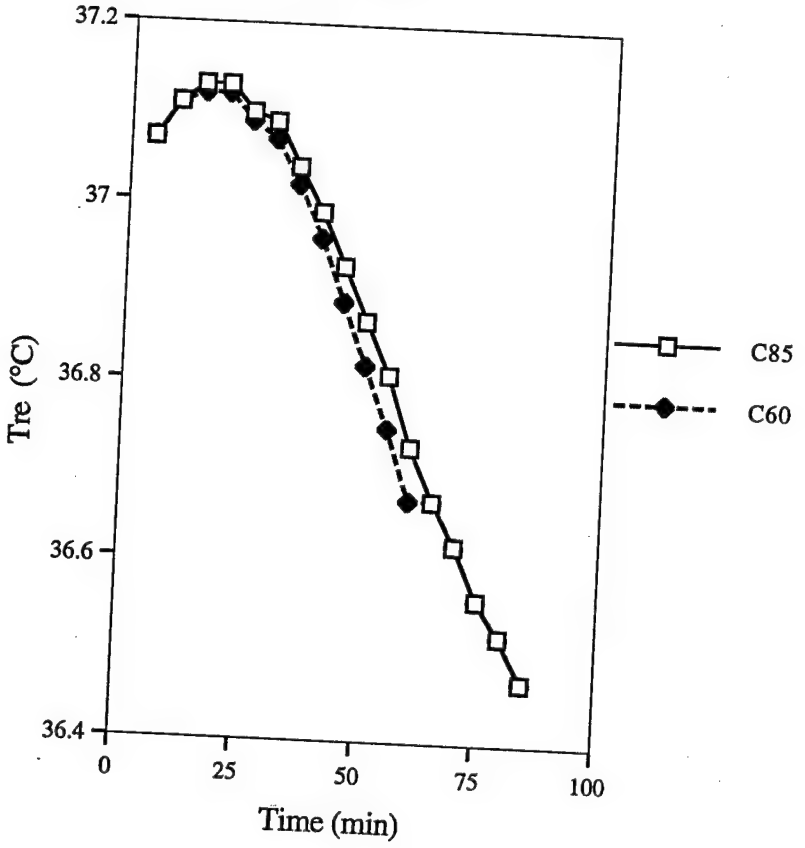

Figure 3. Mean heat storage during the control cold water immersion in C60 and C85.

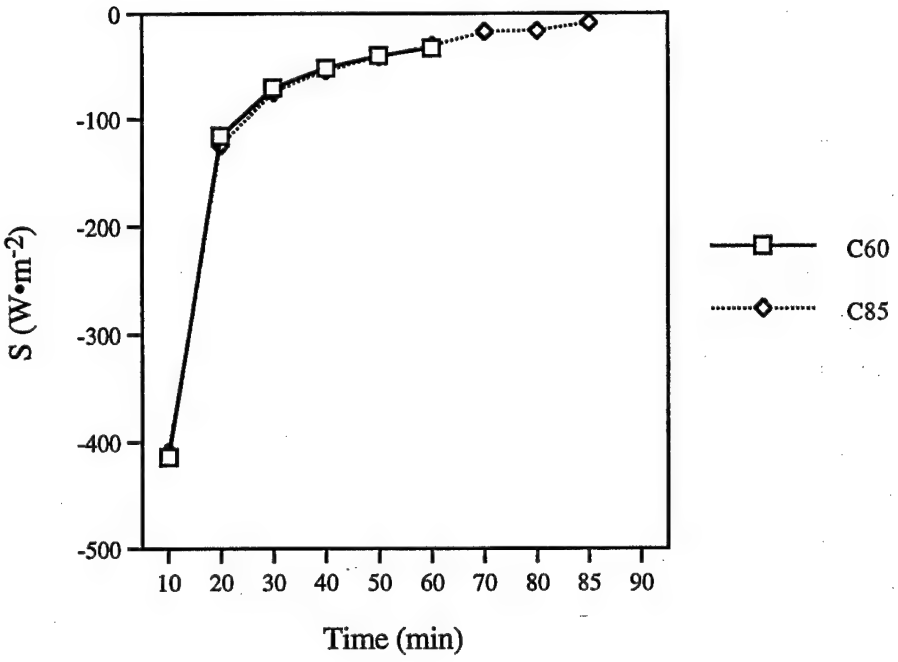

Figure 4. Mean rectal temperature changes during the high CHO, low CHO and control immersions in subjects completing 85 min (T85).

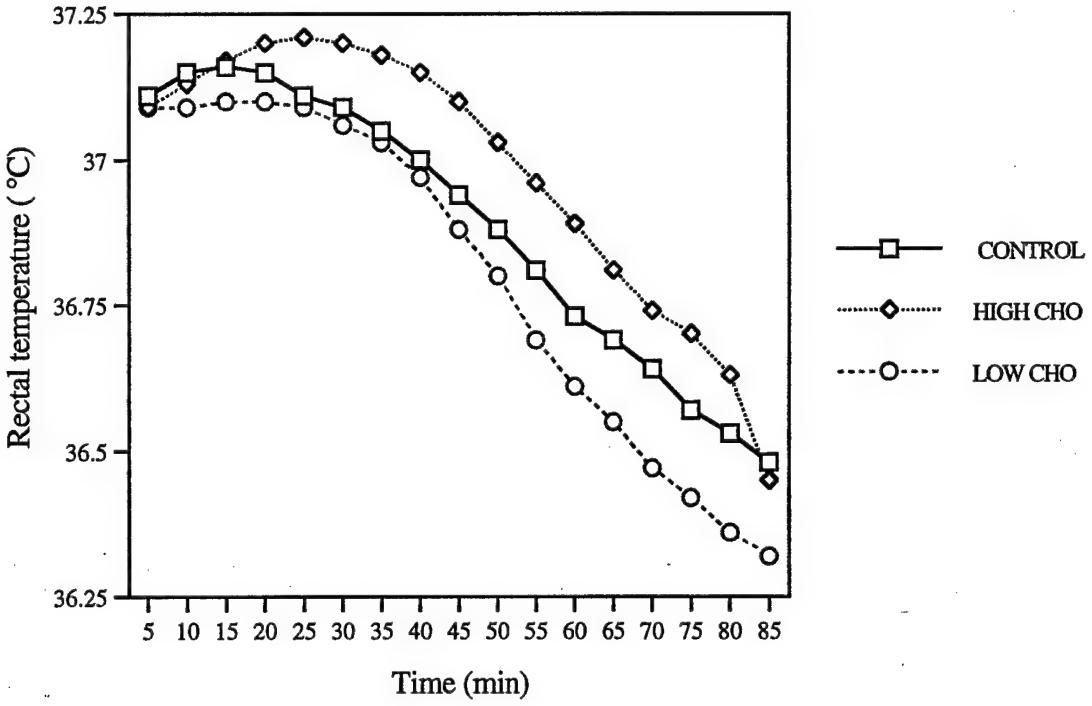

Figure 5. Mean heat storage changes during the three trials of cold water immersion in subjects completing 85 min (T85), n=8.

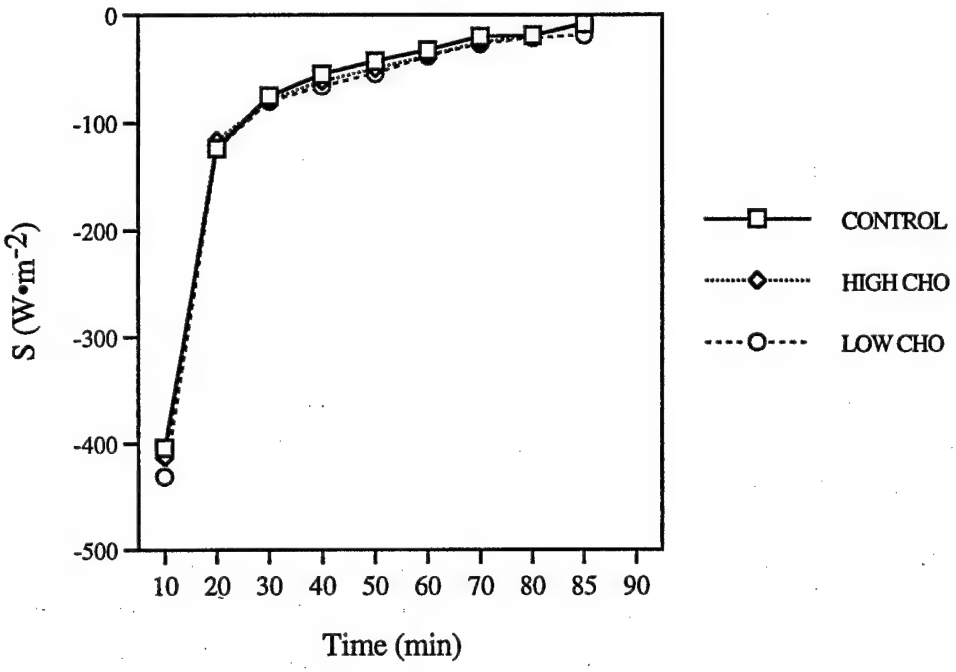

Figure 6. Metabolic rate response to cold water immersion in subjects who completed only 60 or 85 min of immersion.

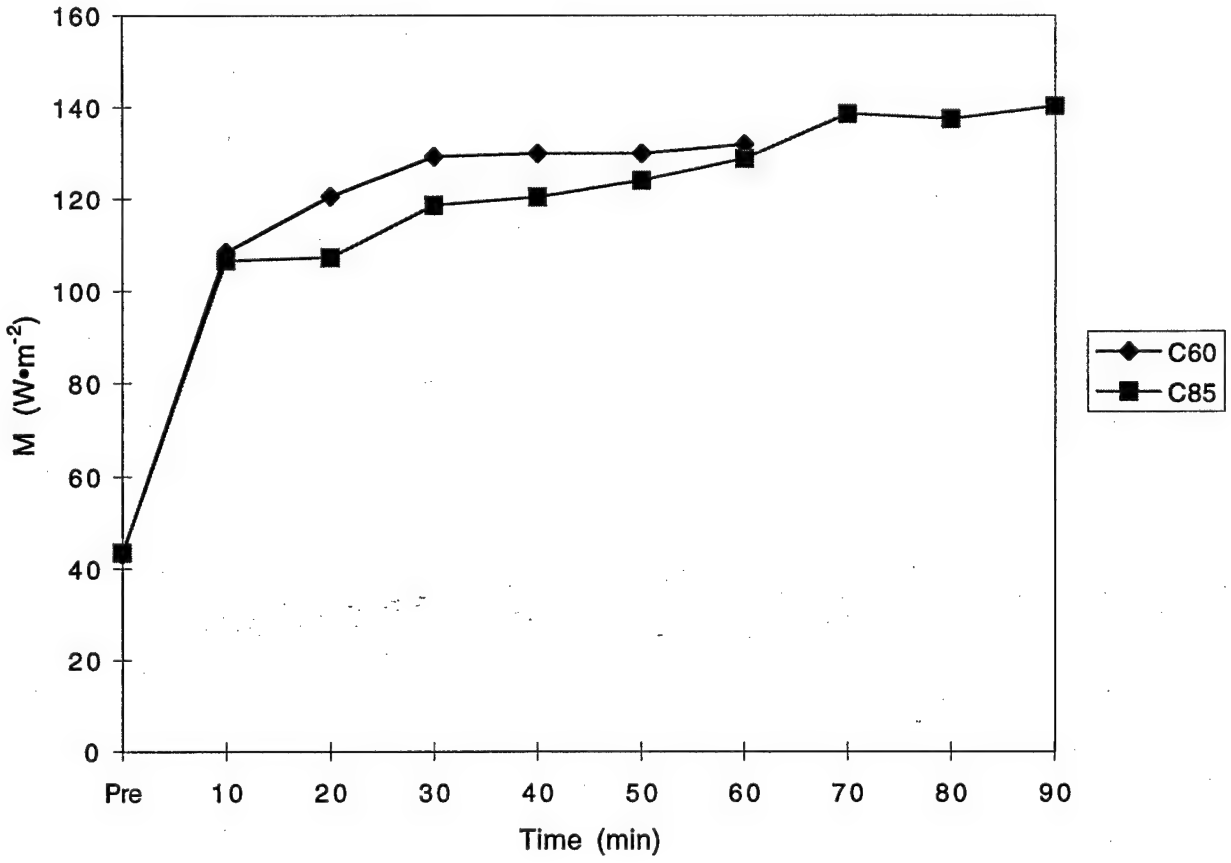

Figure 7. Metabolic rate response during cold water immersion. *indicates significant difference from 60-90 ($p<.005$).

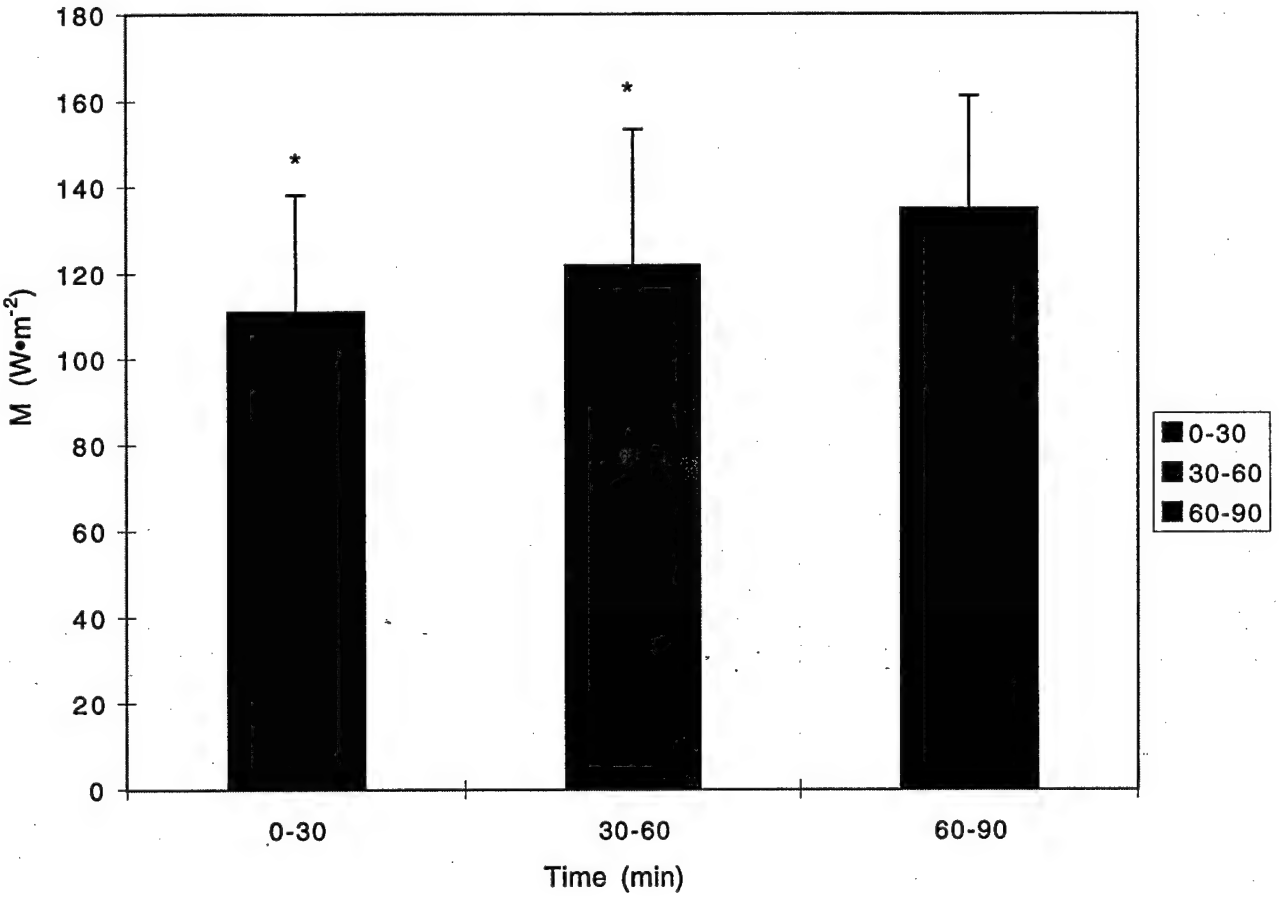

Figure 8. Individual subject data are shown for the peak rate of oxygen consumption during the control cold water immersion, expressed relative to maximal oxygen consumption rate during exhaustive exercise.

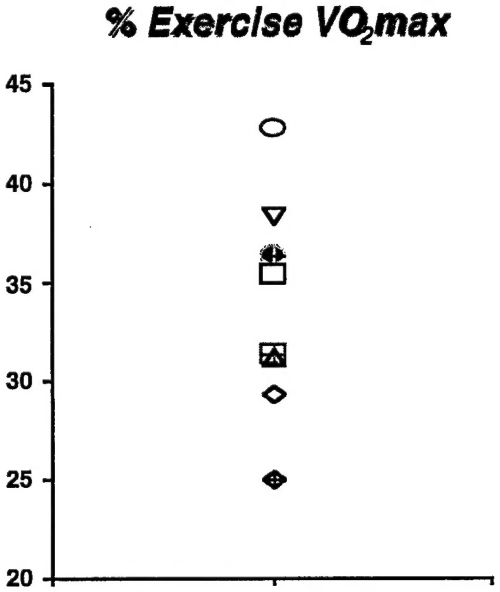

Figure 9. Comparison of metabolic rate during control, high CHO and low CHO immersions

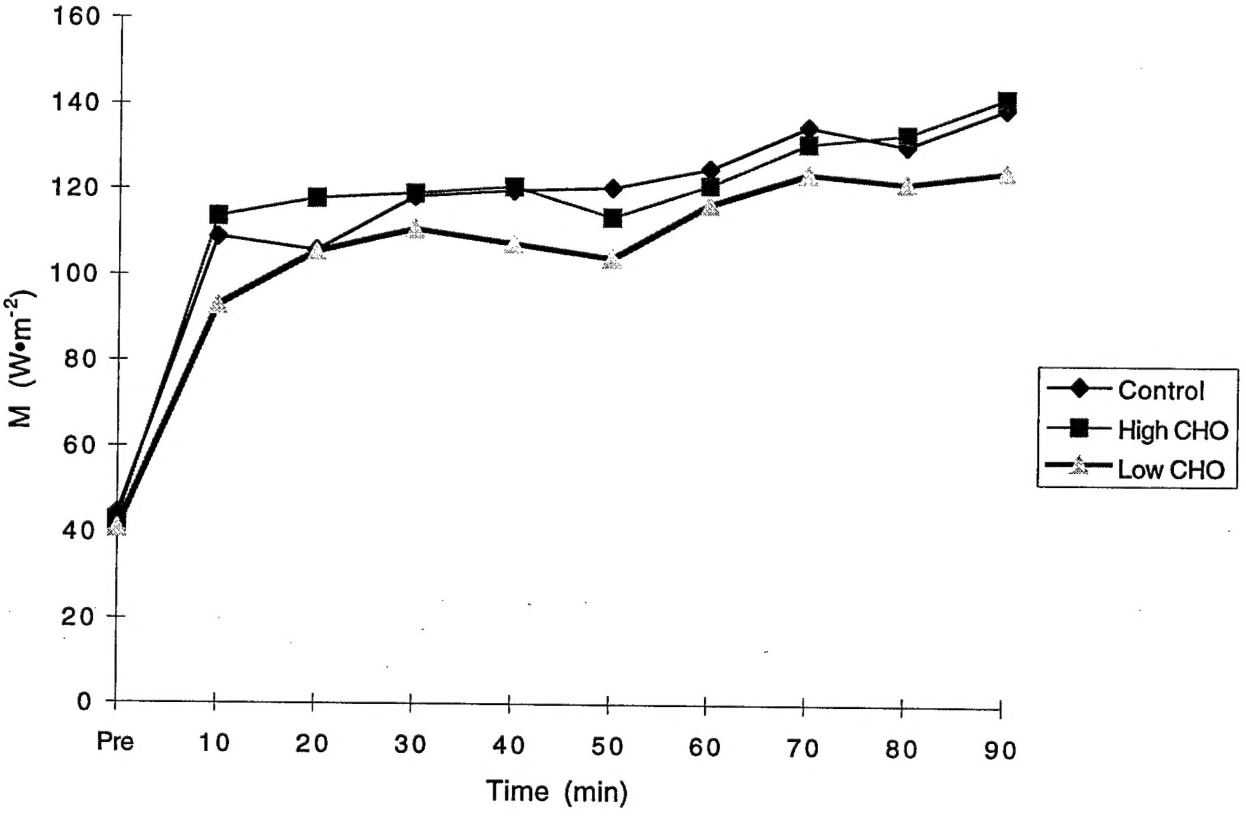

Figure 10. Substrate contribution to M (%M) by carbohydrate (CHO), fat and protein oxidation during control, high and low CHO immersions. *indicates that the low CHO trial was different from both the control and high CHO trials ($p<0.05$).

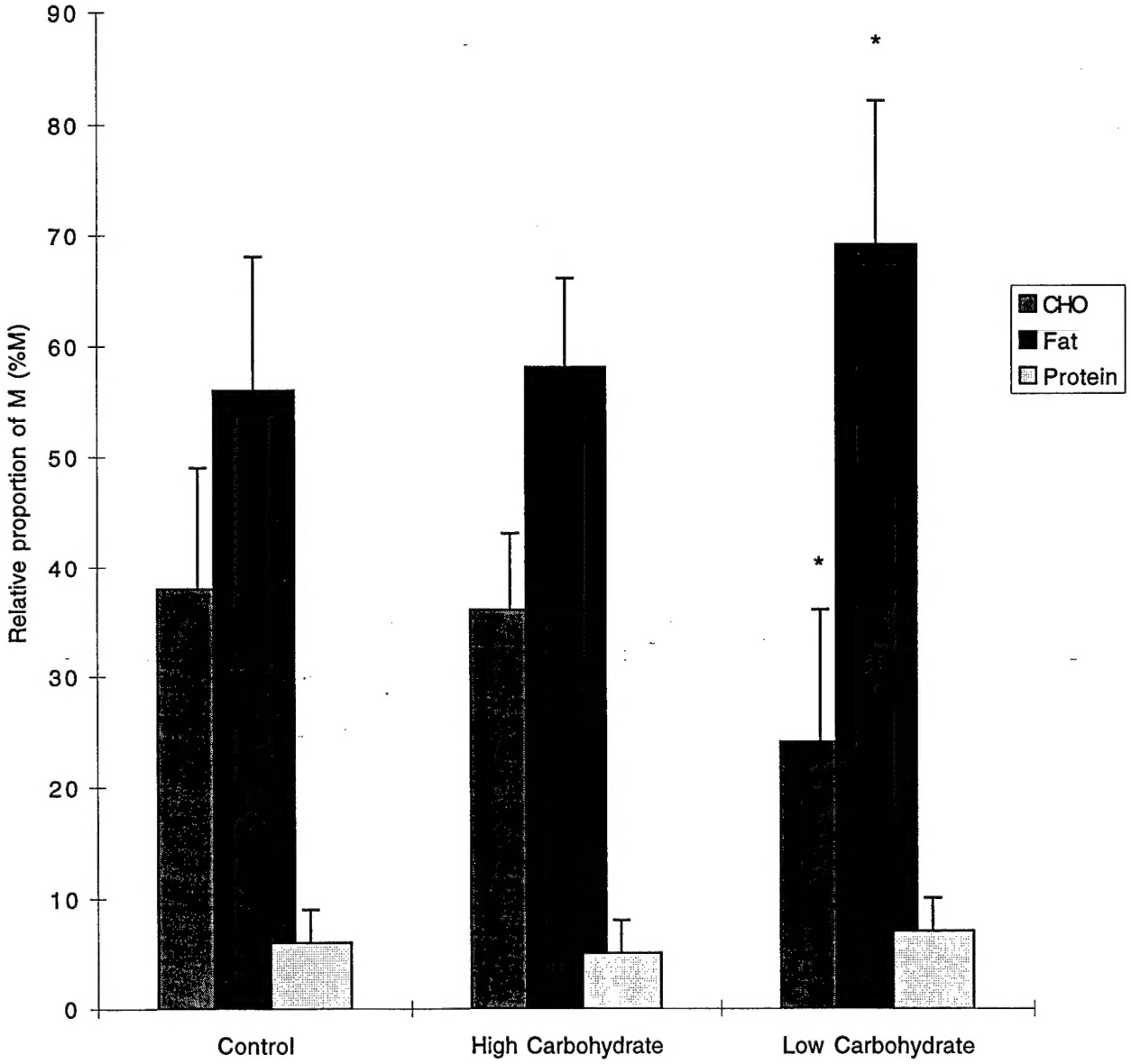

Figure 11. Muscle glycogen content (mean ± SE) before and after control, high CHO and low CHO immersions